177.(New) A transgenic germ cell, obtained from the non-human transgenic vertebrate of Claim 168.

178.(New) A transgenic germ cell, obtained from the progeny non-human transgenic vertebrate of Claim 170.

179.(New) A transgenic non-human vertebrate male germ cell, obtained by a method comprising performing the method of Claim 168; and then collecting male germ cells produced by the transgenic male non-human vertebrate.

180.(New) A transgenic non-human vertebrate male germ cell, obtained by a method comprising performing the method of Claim 170; and then collecting the germ cells produced by the male progeny non-human transgenic vertebrate.

181.(New) Non-human vertebrate semen, comprising the germ cell of Claim 179.

182.(New) Non-human vertebrate semen, comprising the germ cell Claim 180.--.

REMARKS

Applicant's Preliminary Amendment is submitted together with a divisional application directed to the subject matter Claims 24-40, 61-75, 77, 97-111, 113, 127-130, as originally filed in pending parent U.S. Serial No. 09/191,920, which claims were designated Group II in a restriction requirement, mailed March 24, 2000.

The amendment of the title (at page 1, lines 1-3), is to bring these into conformity with the new Claims 135-182.

Applicant believes that no new matter is introduced by any amendments made herein.

At page 1, line 4, Applicant has added continuing data explaining the relationship to U.S. Serial No. 09/191,920 and other divisions and continuations thereof.

Applicant's cancellation of Claims 1-134 is made without prejudice. New Claims 135-182 are added. Support is found, e.g., in Claims 24-40, 61-75, 77, 97-111, 113, 127-130 as originally filed.

In view of the above amendments and remarks, it is submitted that this application is now ready for allowance. If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned attorney at (213) 896-6665.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION:

In the Title, at page 1, lines 1-3, please delete the entire title, and insert therefor:

--TRANSFECTION, STORAGE AND TRANSFER OF MALE GERM CELLS FOR GENERATION OF TRANSGENIC SPECIES [& GENETIC THERAPIES]--.

At page 1, line 4, please delete the entire one-sentence paragraph, and insert the following:

--This application is a division of U.S. Non-provisional Application No. 09/191,920, filed on November 13, 1998, which claims the benefit of U.S. Provisional Application No. 60/065825, filed on November 14, 1997. This application is also related to U.S. Serial No._____, filed on November 12, 2001, U.S. Serial No._____, filed on November 12, 2001, and U.S. Serial No._____, filed on November 12, 2001, which are all divisions of U.S. Serial No. 09/191,920. This application is also related to U.S. Serial No. 09/272,443, filed March 19, 1999, which is a continuation of 09/191,920.--.

At page 4, line 14 through page 15, line 1, please delete the entire paragraph, and insert therefor the following:

--This invention also relates to a novel method for the isolation of spermatogonia, comprising obtaining spermatogonia from a mixed population of testicular cells by extruding the cells from the seminiferous tubules and gentle enzymatic disaggregation. The spermatogonia or stem cells which are to be genetically modified, may be isolated from a mixed cell population by a novel method including the utilization of a promoter sequence, which is only active in cycling spermatogonia stem cell populations, for example, b-Myb or a spermotogonia specific promoter, such as the c-kit promoter region, c-raf-1 promoter, ATM ([ataxaia]ataxia-telangiectasia) promoter, RBM (ribosome binding motif) promoter, DAZ (deleted in azoospermia) promoter, XRCC-1 promoter, HSP 90 (heat shock gene) promoter, or FRMI (from fragile X site) promoter, optionally linked to a reporter construct, for example, the Green Fluorescent Protein Gene (EGFP). These unique promoter sequences drive the expression of the reporter construct only in the cycling spermatogonia. The spermatogonia, thus, are the only cells in the mixed population which will express the reporter construct and they, thus, may be isolated on this basis. In the case of the green

fluorescent reporter construct, the cells may be sorted with the aid of, for example, a FACs scanner set at the appropriate wavelength or they may be selected by chemical methods.--.

At page 10, lines 11-17, please delete the entire paragraph and insert therefor the following:

--"Gene delivery (or transfection) mixture", in the context of this patent, means selected genetic material together with an appropriate vector mixed, for example, with an effective amount of lipid transfect[ion]ing agent. The amount of each component of the mixture is chosen so that the transfection of a specific species of germ cell is optimized. Such optimization requires no more than routine experimentation. The ratio of DNA to lipid is broad, preferably about 1: 1, although other proportions may also be utilized depending on the type of lipid agent and the DNA utilized. This proportion is not crucial.--.

At page 20, lines 15-22, please delete the entire paragraph and insert therefor the following:

--The GFP DNA-transferrin-polylysine viral complexes, prepared as described in Example 4 above, were delivered into the seminiferous tubules of three (3)-week-old B6D2F1 male mice. The DNA delivery by transferrin receptor-mediated endocytosis is described by Schmidt et al. and Wagner et al. (Schmidt et al., Cell 4: 41-51 (1986); Wagner, E., et al. PNAS (1990), (USA) 81: 3410-3414 (1990)). In addition, this delivery system relies on the capacity of adenoviruses to disrupt cell vesicles, such as endosomes and release the contents entrapped therein. The transfection efficiency of this system is almost 2,000 fold higher than lipofection.--.

IN THE CLAIMS:

Please cancel Claims 1-134, without prejudice, as originally filed with parent application 09/191,920, and add the following new Claims 135-182 as being directed to the subject matter of designated claim Group II, which is herein elected.

--135.(New) A non-human transgenic vertebrate produced by the steps of:

(a) administering by injection into a testis of a male non-human vertebrate a transfection mixture comprising at least one polynucleotide encoding a gene product in operable linkage with a promoter, and at least one transfecting agent, other than a liposome/DNA complex, wherein said testis contains the germ cells of the male non-human vertebrate, and wherein said germ

cells are selected from the group consisting of spermatogonial stem cells, type B spermatogonia, primary spermatocytes, preleptotene spermatocytes, leptotene spermatocytes, zygotene spermatocytes, pachytene spermatocytes, secondary spermatocytes, spermatids, and spermatozoa; and

(b) allowing the polynucleotide encoding a gene product to be taken up by, and released into, the germ cells so that the released polynucleotide is incorporated into the genome of the germ cells of said male non-human vertebrate.

136.(New) The non-human transgenic vertebrate of Claim 135, wherein the polynucleotide comprises at least one biologically functional gene.

137.(New) A progeny non-human transgenic vertebrate, carrying in its germ cells at least one xenogeneic polynucleotide sequence, said non-human vertebrate being obtained by further breeding the male non-human vertebrate of Claim 135 with a female of the same species, and selecting the bred progeny non-human transgenic vertebrate for the presence of the transfected xenogeneic polynucleotide.

138.(New) The progeny non-human transgenic vertebrate of Claim 137, being a male comprising native germ cells carrying in their genomes at least one xenogeneic polynucleotide.

139.(New) The non-human transgenic vertebrate of Claim 135, which is selected from the group consisting of mammals and birds.

140.(New) The progeny non-human transgenic vertebrate of Claim 137, which is selected from the group consisting of mammals and birds.

141.(New) The non-human transgenic vertebrate of Claim 135, which is a mammal selected from the group consisting of non-human primates, canines, felines, swine, farm and marine mammals, pachyderms, equines, murine, ovines and bovine, or a bird selected from the group consisting of ducks, geese, turkeys and chickens.

142.(New) The non-human transgenic vertebrate of Claim 135, wherein the mammal is selected from the group consisting of wild and domesticated mammals.

143.(New) The non-human transgenic vertebrate of Claim 135, wherein the mammal is a farm or marine animal.

144.(New) The vertebrate of Claim 135, wherein the mammal is selected from the group consisting of a bull and a pig, and the bird is a chicken.

145.(New) A transgenic germ cell, obtained from the non-human transgenic vertebrate of Claim 135.

146.(New) A transgenic germ cell, obtained from the progeny non-human transgenic vertebrate of Claim 137.

147.(New) A transgenic non-human vertebrate male germ cell, obtained by a method comprising performing the method of Claim 135; and then collecting male germ cells produced by the male non-human vertebrate.

148.(New) A transgenic non-human vertebrate male germ cell, obtained by a method comprising performing the method of Claim 137; and then collecting the germ cells produced by the male progeny non-human transgenic vertebrate.

149.(New) Non-human vertebrate semen, comprising the germ cell of Claim 147.

150.(New) Non-human vertebrate semen, comprising the germ cell Claim 148.

151.(New) A method of producing a non-human vertebrate animal line comprising native germ cells carrying in their genome at least one xenogeneic polynucleotide, comprising

breeding of the progeny non-human transgenic vertebrate of Claim 137, with a member of the opposite sex of the same species; and selecting progeny for the presence of said polynucleotide.

152.(New) A transgenic non-human vertebrate, comprising germ cells carrying in their genomes at least one xenogeneic polynucleotide, said transgenic non-human vertebrate having received an injection in its testis of male germ cells comprising at least one polynucleotide encoding a desired trait or product and at least one polynucleotide encoding a genetic selection marker, said male germ cells comprising the polynucleotide being isolated or selected from a donor male non-human vertebrate with the aid of the selection marker.

153.(New) The transgenic non-human transgenic vertebrate of Claim 152, wherein the polynucleotide comprises at least one biologically functional gene.

154.(New) A progeny non-human transgenic vertebrate, carrying in its germ cells at least one xenogeneic polynucleotide sequence, said non-human vertebrate being obtained by further breeding the male non-human vertebrate of Claim 152 with a female of the same species, and selecting the bred progeny non-human transgenic vertebrate for the presence of the transfected xenogeneic polynucleotide.

155.(New) The progeny non-human transgenic vertebrate of Claim 154, being a male comprising native male germ cells transfected with a xenogeneic polynucleotide.

156.(New) The non-human transgenic vertebrate of Claim 152, which is selected from the group consisting of mammals and birds.

157.(New) The progeny non-human transgenic vertebrate of Claim 154, which is selected from the group consisting of mammals and birds.

158.(New) The non-human transgenic vertebrate of Claim 152, which is a mammal selected from the group consisting of non-human primates, canines, felines, swine, pachyderms, equines, murine, ovines and bovine, or a bird selected from the group consisting of ducks, geese, turkeys and chickens.

159.(New) The non-human transgenic vertebrate of Claim 152, wherein the mammal is selected from the group consisting of wild and domesticated mammals.

160.(New) The non-human transgenic vertebrate of Claim 152, wherein the mammal is a farm or marine animal.

161.(New) The vertebrate of Claim 152, wherein the mammal is selected from the group consisting of a bull and a pig, and the bird is a chicken.

162.(New) A transgenic germ cell, obtained from the non-human transgenic vertebrate of Claim 152.

163.(New) A transgenic germ cell, obtained from the progeny non-human transgenic vertebrate of Claim 154.

164.(New) A transgenic non-human vertebrate male germ cell, obtained by a method comprising performing the method of Claim 152; and then collecting male germ cells produced by the transgenic male non-human vertebrate.

165.(New) A transgenic non-human vertebrate male germ cell, obtained by a method comprising performing the method of Claim 154; and then collecting the germ cells produced by the male progeny non-human transgenic vertebrate.

166.(New) Non-human vertebrate semen, comprising the germ cell of Claim 164.

167.(New) Non-human vertebrate semen, comprising the germ cell Claim 165.

168.(New) A non-human transgenic vertebrate, or its progeny, comprising a native germ cell carrying in its genome at least one xenogeneic polynucleotide, said polynucleotide having been incorporated into the genome of said germ cell through the steps of:

(a) obtaining a maturing male germ cell from a non-human vertebrate;

(b) transfecting the germ cell in vitro with at least one polynucleotide encoding a desired trait in the presence of a gene delivery mixture comprising at least one transfecting agent, and optionally a polynucleotide encoding a genetic selection marker, at about or below the vertebrate's body temperature and for a transfection-effective period of time; and

allowing the polynucleotide encoding a desired trait to be taken up by, and released into the germ cell.

169.(New) The non-human transgenic vertebrate of Claim 168, wherein the polynucleotide comprises at least one biologically functional gene.

170.(New) The progeny non-human transgenic vertebrate of Claim 168, being a male comprising native male germ cells transfected with a xenogeneic polynucleotide.

171.(New) The non-human transgenic vertebrate of Claim 168, which is selected from the group consisting of mammals and birds.

172.(New) The progeny non-human transgenic vertebrate of Claim 170, which is selected from the group consisting of mammals and birds.

173.(New) The non-human transgenic vertebrate of Claim 168, which is a mammal selected from the group consisting of non-human primates, canines, felines, swine, pachyderms, equines, murine, ovines and bovine, or a bird selected from the group consisting of ducks, geese, turkeys and chickens.

174.(New) The non-human transgenic vertebrate of Claim 168, wherein the mammal is selected from the group consisting of wild and domesticated mammals.

175.(New) The non-human transgenic vertebrate of Claim 168, wherein the mammal is a farm or marine animal.

176.(New) The vertebrate of Claim 168, wherein the mammal is selected from the group consisting of a bull and a pig, and the bird is a chicken.

177.(New) A transgenic germ cell, obtained from the non-human transgenic vertebrate of Claim 168.

178.(New) A transgenic germ cell, obtained from the progeny non-human transgenic vertebrate of Claim 170.

179.(New) A transgenic non-human vertebrate male germ cell, obtained by a method comprising performing the method of Claim 168; and then collecting male germ cells produced by the transgenic male non-human vertebrate.

180.(New) A transgenic non-human vertebrate male germ cell, obtained by a method comprising performing the method of Claim 170; and then collecting the germ cells produced by the male progeny non-human transgenic vertebrate.

181.(New) Non-human vertebrate semen, comprising the germ cell of Claim 179.

182.(New) Non-human vertebrate semen, comprising the germ cell Claim 180.--.